Project Report: Subsurface Biospheres

Project Investigators:	**Arthur Spivack , Steven D'Hondt , John Hayes , Kai−Uwe Hinrichs , David Smith , Andreas Teske**

Project Progress

Members of our team at University of Rhode Island (URI) focus on studies of subsurface metabolic activities and biogeochemical fluxes. To further these studies, team members are developing new techniques to study the activity and physiology of energy−limited microbial communities. For example, Bruno Soffientino and Arthur Spivack are working to develop a robust tritium−based method for quantifying metabolic activity at rates that are well below the detection limit of current radiotracer techniques. Other activities include exploratory work on a novel cell extraction method (Kristofer Carlson, Soffientino, David Smith), development of a numerical model to quantify biogeochemical reaction rates at depth (Scott Rutherford, Uri Manor, Guizhi Wang, Steven D'Hondt, and Spivack), analyses of ATP concentrations and dissolved organic compounds in deep subseafloor sediments (Smith, Beverly Chen, Colleen Mouw), and quantification of biogeochemical fluxes and thermodynamic equilibria of biogeochemical reactions (Spivack, Wang, D'Hondt).

Members of our team at University of North Carolina (UNC) Chapel Hill (Andreas Teske, Antje Lauer, Ketil Sørensen, Mark Lever, Karen Lloyd) focus on genetic analyses of subseafloor communities, with a particular emphasis on organic−poor open ocean sediments. These analyses have required development of DNA isolation procedures for geologically complex low−biomass samples. They are positioned near the sensitivity limit for nucleic acid procedures. Lauer, Teske and Sørensen analyzed archaeal communities from subseafloor sediments with the lowest biomass that have been studied to date. Lever and Teske are undertaking functional gene analyses to determine the principal community members associated with specific subseafloor biogeochemical processes. With collaborator Virginia Edgcomb, Teske also explored the environmental stress tolerance of hyperthermophilic vent archaea.

Members of our team at Woods Hole Oceanographic Institution (WHOI) principally focus on organic biogeochemical and isotopic signatures of life in subsurface environments. Kai Hinrichs, Helen Sturt, Kristin Smith, and collaborator Roger Summons, are using analyses of intact polar lipids to identify active prokaryotes at levels of species to orders. Studies by Hinrichs and collaborators are developing an understanding of a ubiquitous, but previously unknown, process, the biological production of ethane and propane in marine sediments. Hayes, Sean Sylva, Sturt, Summons, and Bradley are using organic and isotopic biogeochemistry to document community composition and metabolic structure of the ecosystem in the Lost City Hydrothermal Field. With collaborator Rothman, Hayes and Summons are using isotopic signatures and biogeochemical modeling to improve understanding of the Neoproterozoic carbon cycle.

Highlights

- The hydrogenase assay under development will be the first measure of enzymatic activity applicable to environments with extremely low levels of metabolism. It may provide a robust method for quantifying metabolic activity at rates that are well below the detection limit of current radiotracer techniques (Soffientino et al., 2004).
- We have developed a numerical model that uses profiles of chemical and physical properties to quantify metabolic rates at depth (Manor et al., 2004). Our model is flexible enough to quantify complex variation in rates throughout sediment columns of great depth.
- Our biogeochemical studies demonstrate that metabolic interactions in deep subseafloor ecosystems are complex (D'Hondt et al., in review). Several hypothetically competing processes, including metal reduction, sulfate reduction and methanogenesis, commonly occur in deeply buried sediments. At some sites, aerobic communities live deep beneath anaerobic communities, because the underlying basaltic aquifer supplies oxygen and nitrate to the deep sediments. The sedimentary communities may, in turn, fuel life in the underlying basalts by supplying electron donors and nutrients.
- Sulfur reduction and iron reduction co−occur, with low and relatively constant free−energy yields, in some deep subseafloor sediments (Wang et al., 2004). The organisms that rely on these reactions are traditionally assumed to compete with each other. However, our results suggest that positive feedback between these reactions may help to sustain them.
- The archaeal community in the cold metal−reducing ecosystem of our lowest biomass site includes members of several deeply branching phylogenetic lineages that were previously known only from hydrothermal vents (Sørensen et al., 2004). This conspicuous occurrence pattern suggests that cold subsurface sediments may harbor seed populations of vent systems.
- Three archaeal lineages are common in subseafloor sediments throughout much of the world ocean (Lauer et al., 2004). Two of these lineages (Deep−Sea Archaeal Group and Marine Benthic Group A) occur selectively in marine sedimentary habitats. Unexpectedly, known methanogens are scarce in subseafloor sediments, although methane production clearly occurs in subseafloor sediments throughout the world ocean.

- In deeply buried methane/sulfate transition zones of the Peru Margin, we have found intact polar lipid types previously only found in *Sulfolobus spp*. (Sturt et al., 2004). These observations raise the possibility that Crenarchaea are involved in subseafloor anaerobic oxidation of methane.
- Our isotopic and bioenergetic studies suggest that ethane and propane are commonly produced in marine sediments by biogeochemical reactions in which acetate serves as electron acceptor (Hinrichs et al., submitted). These reactions proceed close to the biological energy quantum and provide a novel sink for major products of fermentation.
- Many biomarker lipids from the H_2–rich, Lost City Hydrothermal Field are marked by extraordinary enrichment of ^{13}C (Kelley al., submitted). The pattern is that expected in systems in which autotrophs flourish so strongly that seawater DIC becomes the limiting nutrient.
- Biogeochemical principles and isotopic records both suggest that concentrations of dissolved organic carbon in the Neoproterozoic ocean were probably high and that surging flows of carbon into and out of that reservoir were a key feature of the Neoproterozoic CO_2 and redox balances (Rothman et al., 2003).

Roadmap Objectives

- ***Objective No. 2.1:*** Mars exploration
- ***Objective No. 2.2:*** Outer Solar System exploration
- ***Objective No. 4.3:*** Effects of extraterrestrial events upon the biosphere
- ***Objective No. 5.2:*** Co–evolution of microbial communities
- ***Objective No. 5.3:*** Biochemical adaptation to extreme environments
- ***Objective No. 6.1:*** Environmental changes and the cycling of elements by the biota, communities, and ecosystems
- ***Objective No. 6.2:*** Adaptation and evolution of live beyond Earth
- ***Objective No. 7.1:*** Biosignatures to be sought in Solar System materials
- ***Objective No. 7.2:*** Biosignatures to be sought in nearby planetary systems

Mission Involvement

Mission Class*	***Mission Name (for class 1 or 2) OR Concept (for class 3)***	***Type of Involvement*****
2	MSL	Background Research
3	Future missions to Mars, Europa, and other planetary bodies.	Research or Analysis Techniques

* Mission Class: Select 1 of 3 Mission Class types below to classify your project:
1. Now flying OR Funded & in development (e.g., Mars Odyssey, MER 2003, Kepler)
2. Named mission under study / in development, but not yet funded (e.g., TPF,

Mars Lander 2009)
3. Long–lead future mission / societal issues (e.g., far–future Mars or Europa, biomarkers, life definition)

** Type of Involvement = Role / Relationship with Mission
Specify one (or more) of the following: PI, Co–I, Science Team member, planning support, data analysis, background research, instrument/payload development, research or analysis techniques, other (specify).

Results from our subseafloor studies, such as the thermodynamically balanced co–occurrence of hypothetically competing iron and sulfate reduction, provide models for possible life on Mars. Consequently, they can help to inform scientific planning for the MSL mission and future missions to other planetary bodies.

Techniques that we are developing (such as the tritium assay of hydrogenase activity) and applying (such as the thermodynamic assessment of metabolic reactions) will ultimately be useful for testing and quantifying the occurrence of metabolic activity and for testing the occurrence of specific metabolic pathways on other planetary bodies.

Field Expeditions

Field Trip Name: IODP Leg 301

Start Date: June	***End Date:*** July
Continent: eastern Pacific Ocean	***Country:*** U.S.A.
State/Province:	***Nearest City/Town:*** Astoria, Oregon
Latitude: 48 degrees N	***Longitude:*** 128 degrees W
Name of site(cave, mine, e.g.): Juan de Fuca Ridge	***Keywords:*** Subsurface, mid–ocean ridge, prokaryote

Description of Work: Team member Mark Lever (UNC) sailed as a microbiologist on the first Integrated Ocean Drilling Program expedition (Leg 301, Juan de Fuca Ridge) to study life in relatively hot sediments that overlie young basalt, and to study life in the hot (70–80 degree C) basaltic aquifer. Collaborators Verena Heuer (organic biogeochemist) and Fumio Inagaki (microbiologist) also sailed on the expedition.

Members Involved:

Cross Team Collaborations

With Virginia Edgcomb (NAI Postdoctoral Fellow at the Marine Biological Laboratory), URI Team member Andreas Teske is working to define physiological adaptations or incompatibilities of vent archaea for growth or survival under vent and subsurface regimes. Recent results are described by

Edgcomb et al. (2004) and briefly outlined in the Executive Summary of this annual report.

With Victoria Orphan (NASA Ames Research Center NAI Team) and Christopher House (Pennsylvania State University NAI Team), URI Team member Kai Hinrichs is studying the microbiology and biogeochemistry of anaerobic methane–oxidizing communities at seafloor methane seeps. Results to date include several publications, most recently Orphan et al. (2004).

With Christopher House (Pennsylvania State University NAI Team), URI Team members are studying the microbiology and biogeochemistry of anaerobic methane–oxidizing communities in subseafloor environments of the eastern Pacific Ocean.